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Wavelength dependence of the optomotor response in zebrafish (*Danio rerio*)

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Abstract

The action spectrum of motion detection in zebrafish (*Danio rerio*) was measured using the optomotor response in the light adapted state. The function has a single maximum at 550–600 nm, and is similar to the spectral sensitivity function of the L-cone type in the mid and long wavelength range. At shorter wavelengths the values of three of the five fish tested are lower. As in goldfish [Vis. Res. 36 (1996) 4025], the result indicates a dominance of the L-cone type with an inhibitory influence of M- or S-cones. Experiments with a red/green striped cylinder showed that the optomotor response was at minimum whenever the L-cone type was not modulated by the moving pattern. This demonstrates that motion vision in zebrafish is "color blind", using mainly one of the four cone types probably involved in color vision.

Keywords: Motion; Color vision; Zebrafish (*Danio rerio*); Optomotor response

1. Introduction

Many animals exhibit an optomotor response whenever a moving stimulus, e.g. a rotating striped cylinder, covers large parts of the visual field. The optomotor response consists of eye, head or whole body movements, and helps to compensate movements of the environment and to stabilize its image on the retina. This is important for animals in their natural habitat, for example in the context of course control in flying insects (Egelhaaf, Hausen, Reichardt, & Wehrhahn, 1988) or in orientation of fish in flowing water (Lyon, 1904). The optomotor response was used to study the mechanisms of motion vision, as in the classical experiments with the beetle *Chlorophanus* which are the basis of the Hassenstein–Reichhardt model of motion detection (Hassenstein & Reichardt, 1956). However, the optomotor response was also used as a test for color vision in early experiments. Schlieper (1927) investigated a number of species in insects, crustaceans, and even vertebrates (a lizard) by using rotating drums with alternating colored and gray stripes. In all investigated species he found a combination of color and a shade of gray at which the optomotor response came to zero. It seemed that these species are color blind until Schlieper tested honeybees. To his surprise, they reacted in the same way, despite of the fact that they possess an excellent color vision as shown in training experiments by von Frisch (1914). Therefore, the conclusion was now that not the animal as such, but the optomotor response is "color blind". A measurement of the action spectrum of the optomotor response in the honeybee by Kaiser and Liske (1974) revealed a single maximum at about 540 nm which fitted the spectral sensitivity function of the "green" retinula cells. Thus, honeybees use one photoreceptor type only for motion detection which explains the color blindness of this behavior.

Measurements of the action spectrum of the optomotor response in several vertebrate species revealed a single maximum in the long wavelength range (frog: Birukow, 1950; goldfish and tadpole: Cronly-Dillon & Muntz, 1965; goldfish and turtle: Schaerer, 1993; Schaerer & Neumeyer, 1996). In goldfish and turtle the maximum is located around 650 nm, and indicates a dominance of the L-cone type. Only in goldfish it was shown that motion vision is indeed "color-blind" (Schaerer & Neumeyer, 1996). This was the case whenever the red and green stripes of a moving cylinder did not modulate the excitation of the L-cone type.

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The aim of this study in zebrafish was twofold: (1) It should be shown whether the "color-blindness" of the optomotor response can be demonstrated in another fish species. This would further corroborate the idea that this is a general principle realized in visual systems in more general. (2) The zebrafish (*Danio rerio*) was chosen as a subject because of its importance in vertebrate genetics and development. A large number of mutants has been isolated until now, including some in which the visual system is being affected (Brockerhoff et al., 1995; Neuhauss et al., 1999). We hope that mutants with known defects or aberrations in the retina can be used to understand the neural basis of visual functions. This, however, requires a profound knowledge of the wild-type. Optomotor response and optomotor nystagmus are behaviors which can be applied in zebrafish rather easily (Bilotta & Saszik, 2001; Li, 2001).

In zebrafish four morphologically different cone types have been described by Branchek and Bremiller (1984) with maximal sensitivity at 360, 417, 480 and 570 nm (Nawrocki, Bremiller, Streisinger, & Kaplan, 1985; Robinson, Schmitt, Hárosi, Reece, & Dowling, 1993). The rods show maximal sensitvity at 501 nm (Nawrocki et al., 1985). In our investigations the contribution of the different cone types to motion detection was derived from a comparison between the action spectrum of the optomotor response and the cone spectral sensitivity functions. The "color blindness" of motion vision is demonstrated by using colored striped drums.

2. Material and methods

2.1. Animals

Adult zebrafish were obtained from local dealers. They were of normal shape and had a length of 3–4 cm. Both males and females were used. The fish where kept in 12 or 25 l tanks under a 12/12 h light/dark rhythm. The home tanks were illuminated by fluorescent tubes (Osram L36 W/12 daylight, 70 kHz by Osram Quicktronic electronic control gear). The flickerfree light, obtained by using the control gear, seems to prevent the rather erratic swimming behavior of zebrafish and guppies sometimes observed when kept under fluorescent tubes driven with 50 Hz (John Endler, CV-net communication). The average water temperature was 23 °C. For the experiments the fish were transferred from their home tanks into the circular test tank of the apparatus.

2.2. Setup

The optomotor response was measured in the setup shown in Fig. 1. The apparatus consisted of a stationary cylindric test tank (11 cm diameter, 13 cm height) made from Plexiglass in which the fish could swim freely. In the centre of the test tank there was a vertical plastic rod (diameter 2.5 cm, 13 cm height) to provide swimming parallel to the tank wall. The tank was concentrically surrounded by a cylinder (diameter 14 cm) consisting of stripes made of white cardboard and equally wide slits. A black velvet curtain surrounded this cylinder at a distance of 20 cm, and provided high contrast. The striped cylinder was placed on a rotatable Plexiglass disk which was turned by a motor (Faulhaber). It could be rotated in both directions and at various speeds.

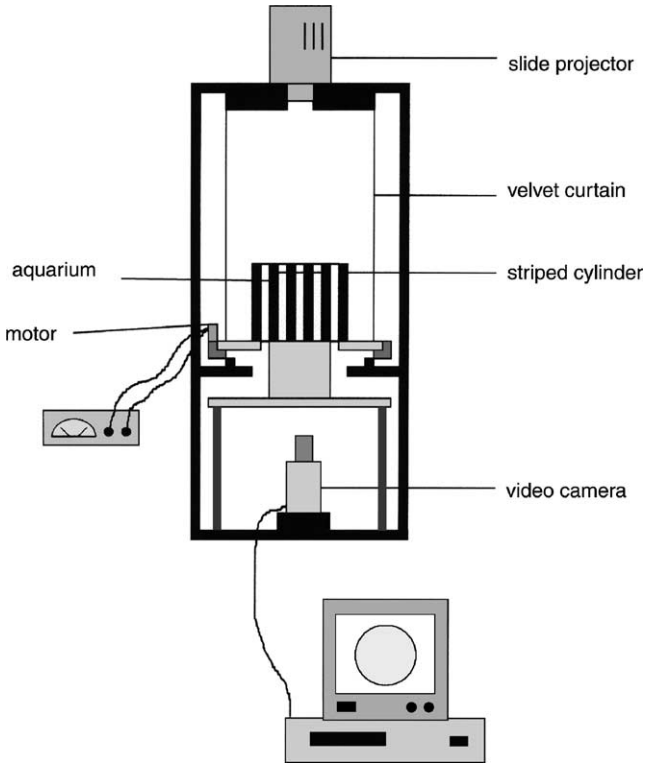

Fig. 1. Setup for the measurement of the optomotor response (see text).

A slide projector (Leitz Prado Universal, 250 W, 220 V) illuminated the white stripes of the cylinder and the test tank from above. Quasi-monochromatic light was obtained by using interference filters (Schott & Gen, type DIL, half-band width: 8–14 nm). Neutral density filters (Schott & Gen, type NG) were used to attenuate intensity. The filters were inserted in a filter chamber of the slide projector. The spectral range between 416 and 699 nm was investigated in steps of 15–20 nm. Measurements in the ultraviolet range of the spectrum were not performed.

The behavior of the fish was monitored from below by a video camera (Burle Video Kamera TC 654 EAX) and recorded on a VCR (Panasonic AG-6124). The fish was observed simultaneously on a monitor (Panasonic BT-D2020 PY). In the first experiments we tested different parameters of the moving stimulus. Two different card-

board cylinders were used, one with 1 cm, the other with 2 cm wide stripes and slits, respectively. Seven different pattern velocities: 6, 8, 10, 12, 14, 18 and 20 rounds per minute (rpm), were tested. In the main experiments we used a "standard" cylinder with 2 cm wide stripes and slits, respectively, rotating at a velocity of 10 rpm (60°/s).

For the experiments in which the "color blindness" of the optomotor response was tested, a cylinder with alternating red and green stripes (each 2 cm wide) was used. Red and green cardboard were selected after measuring their spectral reflectance (Instrument Systems, Spectro 100). The red cardboard reflected mainly (30–90%) above 600 nm, and less than 10% at shorter wavelengths. The green cardboard had a high reflectance between 470 and 570 nm (30–80%). In this experiment the setup was illuminated by two slide projectors, each equipped with an interference filter, 490 and 630 nm, respectively. The light of the second slide projector was directed onto the striped cylinder via a mirror, so that an additive mixture of the colored light was obtained.

2.3. Light measurement

The intensity of the monochromatic light reflected by the white cylinder was measured with a radiometer/photometer (EG & G, IL 1700) in W/cm^2, and converted by calculation into amount of quanta/cm^2 s. The detector head of the radiometer was directed towards a white cardboard (5×5 cm) at the position of the striped cylinder. The full white light of the slide projector impinging the surface of the tank during the pauses (necessary to keep the fish in the light-adapted state) was measured photometrically and gave values of about 1700 lx.

2.4. Procedure

2.4.1. Measurement of the action spectrum

The procedure was very similar to that of our previous experiments in goldfish and is described there in detail (Schaerer & Neumeyer, 1996). At the beginning of an experimental session a fish was transferred into the circular test tank of the setup surrounded by the stationary, striped cylinder, and adapted to white light (1700 lx) for 5 min. Then the white light was replaced by the monochromatic light, and the motor was started to rotate the pattern. The recording of the optomotor response started 20 s later, to avoid the startle behavior many fish showed at beginning of pattern movement. After this delay the optomotor response was recorded for 1 min. Then, the rotation of the cylinder stopped, and white adaptation light was given for 2 min, before the next test period of 1 min duration started. Here, the wavelength of the monochromatic light was the same, but its intensity was reduced in steps of half a log unit. This continued until no optomotor response was observed. Then, the next wavelength was chosen (at random). One experimental session per day was performed with each fish which lasted for about 1 h. During this session only one direction of cylinder motion was tested, the opposite direction was shown at the next day. This yielded more reliable results than changing the direction of pattern movement between trials.

2.4.2. Measurement of the optomotor response with the red–green cylinder

The red–green cylinder was illuminated from above by two slide projectors. The two wavelengths 490 and 630 nm were given simultaneously. The intensity of one of the two monochromatic lights was kept constant, while the intensity of the second one was reduced in steps of 0.25 log units. The reactions of the fish were recorded for 1 min. The time between two stimulus presentations was 2 min, during this time white light was given.

2.4.3. Data acquisition and analysis

The optomotor response in zebrafish is more difficult to investigate than in goldfish. The reason is that zebrafish are very fast and active swimmers. Even in a stationary surrounding (at the beginning of the experimental sessions) zebrafish are often swimming which is not so much the case with goldfish. Pattern movement sometimes does not change the spontaneous swimming activity, sometimes only in part. As a result, the data within each fish, but also between fish are more scattered than desirable. Only after introducing the central post in the test tank and using flickerfree light above their home tanks reliable results were obtained.

The fish followed the rotating striped cylinder by swimming along the wall of the tank. The optomotor response was quantified as "optomotor gain" which was obtained as follows: during one minute we counted how many rounds the fish was swimming with (+) and against (−) the pattern. The difference divided by the number of rotations of the pattern in one minute was defined as optomotor gain which is optimal at a value equal to 1. A gain of 0.6 was chosen as a threshold criterion for motion detection. This value was derived in preliminary experiments showing that a swimming rate of 6 rpm at a pattern velocity of 10 rpm is significantly higher than the spontaneous swimming behavior of the fish. In the diagrams, the mean values of the optomotor gain obtained with clockwise and counter-clockwise pattern movement are shown.

3. Results

3.1. Spontaneous and pattern-induced swimming activity

The spontaneous activity of the fish, i.e. the activity with a non-moving striped cylinder (2 cm wide stripes

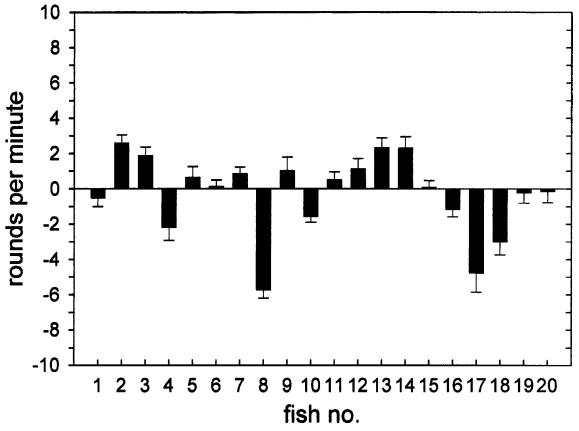

Fig. 2. Spontaneous swimming behavior of 20 zebrafish with stationary striped cylinder. Positive values: clockwise, negative values counter-clockwise swimming.

and slits), was determined under white light of high intensity. The results are shown for 20 fish in Fig. 2. Individual fish showed very different activities, most of them swam in total between 0 and 3 rpm, only two fish (Nos. 8 and 17) swam more. The results are mean values from several measurements (n between 20 and 42 for the individual fish). They indicate spontaneous preferences of swimming directions. Here, positive values indicate clockwise, negative values counter-clockwise swimming.

In order to determine the optimal stimulus parameters for the optomotor response of the zebrafish, different pattern velocities and stripe widths were tested under white light. Pattern velocities of 6, 8, 10, 12, 14, 18 and 20 rpm (i.e. 36, 48, 60, 72, 84, 96, 108, 120°/s) and two cylinders with 1 or 2 cm wide stripes and slits, respectively, were used. The average data for 20 fish are shown in Fig. 3. The total number of rounds within a test minute increased with rising speed of the pattern. At low pattern velocities (6, 8, and 10 rpm) the fish followed the pattern almost optimal. At higher velocities (12, 14 and 18 rpm) the fish swam slightly slower, and at 20 rpm faster than the pattern moved. The widths of the stripes obviously had no influence on the swimming behavior. On the basis of these results we decided to use the following stimulus parameters: 10 rpm pattern velocity, and 2 cm width of stripes and slits, respectively.

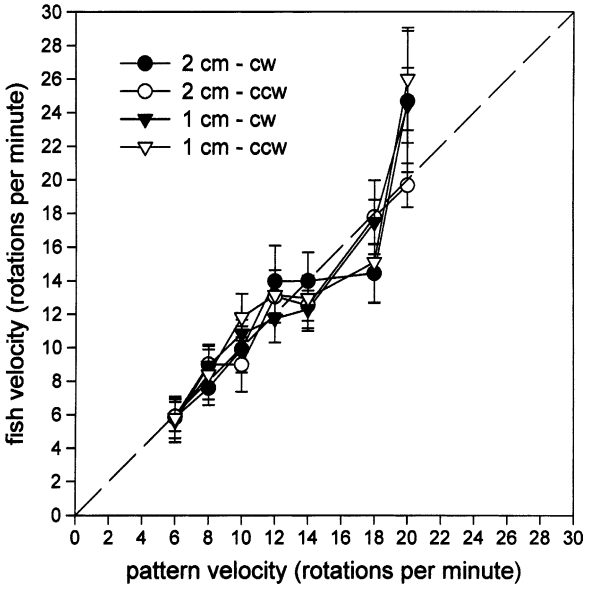

Fig. 3. Optomotor response tested with 1 and 2 cm stripe and slit widths, and seven pattern velocities. Mean values of 20 zebrafish.

3.2. The action spectrum of the optomotor response

Eleven wavelengths in the spectral range from 416 to 699 nm were tested in five light-adapted zebrafish. In Fig. 4 the results of fish N1 and N2 are shown as examples. With fish N1, the threshold response (gain 0.6) was not reached when the shortest wavelengths 416 and 443 nm were tested, even not at the highest intensities.

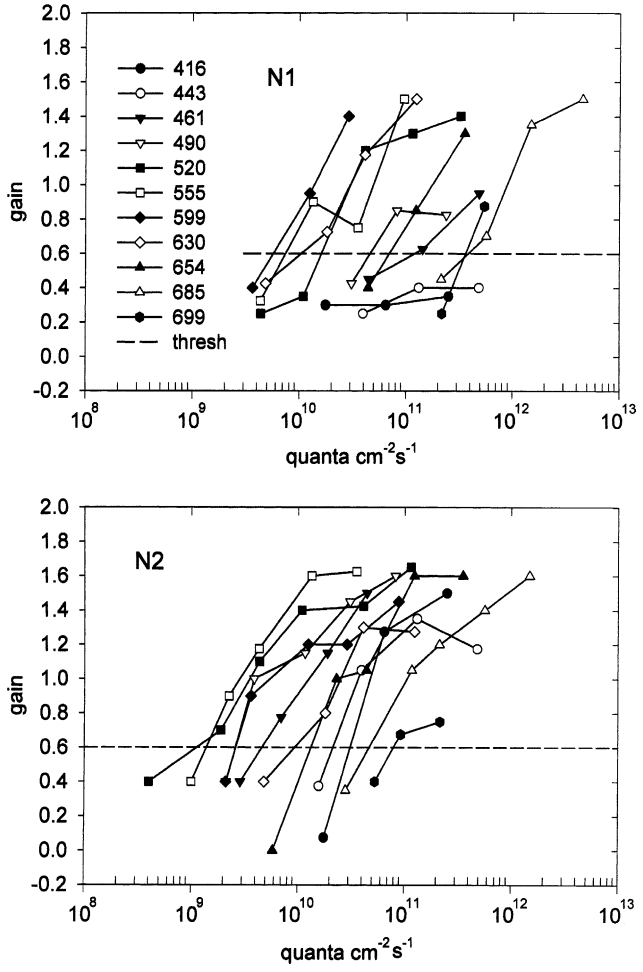

Fig. 4. Optomotor response (gain) of two fish, N1 and N2, as an example. Abscissa: amount of quanta/cm^2 s reflected by the white stripes of the cylinder for wavelengths between 416 and 630 nm (parameter of the curves). Gain = 0.6: threshold criterion.

At longer wavelengths the optomotor gain increased with light intensity. The lowest amount of quanta/cm^2 s to reach threshold was found at 599 nm (N1), and at 555/520 nm (N2), indicating highest sensitivity. With 699 nm the highest amount of quanta/cm^2 s was necessary. To obtain the action spectrum for each of the five fish, the amount of quanta/cm^2 s at threshold was read out of the diagrams. The results are shown in Fig. 5 as relative spectral sensitivity functions. Here, the maximal sensitivity values of each fish were normalized to a value equal to 1. The absolute values of quanta/cm^2 s at maximum were the following: 1.1×10^9 (fish N2), 1.7×10^9 (fish P), 5.8×10^9 (fish N1), 2.7×10^9 (fish T), and 1.1×10^{10} (fish R). The results are shown together with the spectral sensitivity function of the L-cone type (dashed line, after Palacios et al., 1996). Despite the fact that the data are rather scattered, the action spectrum reveales a single maximum between 550 and 600 nm. The comparison with the L-cone spectral sensitivity function which is at maximum at 570 nm indicates that the long wavelength flank follows the L-cone sensitivity function. In the short wavelength range, however, the values between 416 and 520 nm are for three of the five fish below the L-cone function. The short wavelength flank is steeper, and the scatter is in the range of about 1 log unit. The fact that there is one maximum only, indicates a dominance of the L-cone type. The result is similar to that in goldfish (Schaerer & Neumeyer, 1996).

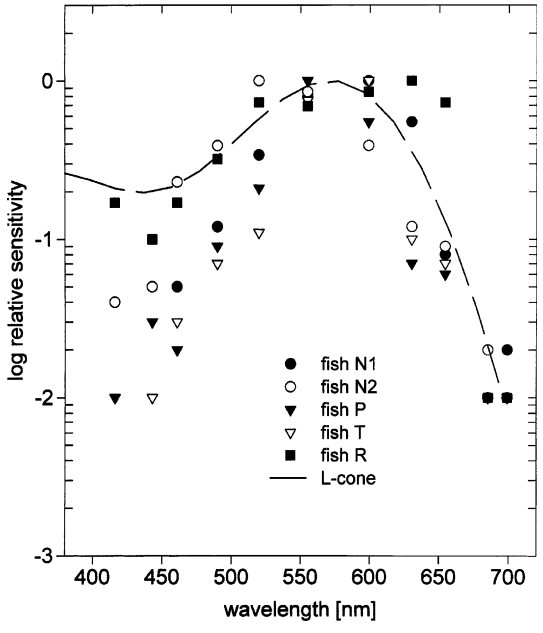

Fig. 5. Relative spectral sensitivity of five goldfish measured with the optomotor response. The absolute amount of quanta/cm^2 s for each wavelength necessary to reach the threshold criterion of gain = 0.6 was read out of Fig. 4 and corresponding diagrams. Dashed line: spectral sensitivity of the L-cone type (after Palacios, Goldsmith, & Bernard, 1996).

3.3. Measurement of the optomotor response with the red–green cylinder

If motion vision measured with the optomotor response is dominated by the L-cone type as indicated by Fig. 5, the zebrafish should behave "color blind" under our experimental conditions. This can be tested by using a colored cylinder with red and green stripes. Moving this cylinder, the zebrafish should see motion only when the excitation of the L-cone type is temporally modulated, but not when the red and green stripes are "equiluminant" for this cone type. We tested this prediction by applying the method of silent substitution (Estévez & Spekreijse, 1982). By illuminating the red–green cardboard cylinder simultaneously with two monochromatic lights (490 and 630 nm) which were independently adjustable in intensity, there should be one intensity ratio at which the L-cone type is equally stimulated by the red and green stripes. In this case the fish should not see motion and, thus, should not follow the pattern.

In a first step the cylinder was illuminated with only one of the two wavelengths to be certain about the amount of quanta/cm^2 s necessary for each fish to show a response above threshold. The results are shown in Fig. 6. For illumination with 490 nm the absolute values at threshold (gain 0.6) ranged from 2.0×10^{10} to 3.1×10^{11} and for illumination with 630 nm from 2.1×10^{10} to 1.2×10^{11} quanta/cm^2 s for individual fish.

In the actual tests, the amount of quanta of one of the two monochromatic lights was kept constant at a value above threshold, and the second monochromatic light was added in different (above-threshold) intensities. The results are shown in Fig. 7 for six of the seven fish tested. Here the maximal optomotor response of each fish was set at 100% and all other data points were plotted as percentage of the maximal reaction. In the same figure, the spontaneous activity is shown obtained with a stationary red–green cylinder illuminated with 490 or 630 nm light (dashed or dotted line, respectively). To give an example: when 490 nm was kept constant (filled symbols) at 2.71×10^{11} quanta/cm^2 s, fish R showed a minimal reaction when 630 nm was given at an intensity of 5.0×10^{11} quanta/cm^2 s. In this case the optomotor response declined from 100% to 35%. When 630 nm was fixed at 2.32×10^{11} quanta/cm^2 s (open symbols), the reaction of fish R was minimal when the 430 nm light was added at an intensity of 1.0×10^{11} quanta/cm^2 s. A decline of the reaction from 100% to 30% could be registered. Similar results are shown in Fig. 7 for fish G. In both fish the optomotor response at minimum is in the range of spontaneous activity. Each of the seven fish was tested twice when the light of 490 nm was constant and twice when the light of 630 nm was fixed. In all cases a certain intensity ratio of the two monochromatic lights was found at which the optomotor response was reduced (Table 1).

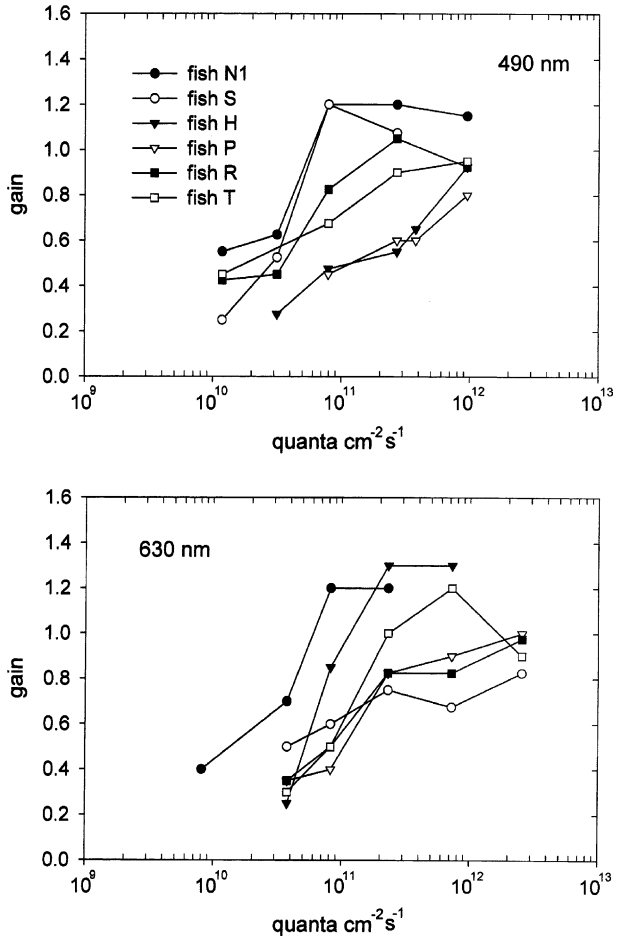

Fig. 6. Optomotor response (gain) for two wavelengths, 490 nm (above) and 630 nm (below), illuminating the red/green cylinder (see text). Abszissa: amount of quanta/cm^2 s measured at white cardboard. Parameter of the curves: individual fish.

3.4. Calculation of M- and L-cone modulation under the condition of minimal optomotor response

To calculate the modulation in the excitation of the L- and M-cone type, elicited by the red and green stripes, respectively, we determined the amount of quanta/cm^2 s of both monochromatic lights at the response minimum in Fig. 7 and the other diagrams (not shown). The values, listed in Table 1, correspond to the amount of quanta/cm^2 s reflected by the white cardboard which showed a constant reflectance of 0.95 between 450 and 700 nm. To obtain the amount of quanta of the monochromatic light reflected by the red and green stripes, these values were multiplied by the relative reflectance of the red and the green cardboard, respectively, at the corresponding wavelengths (for the red cardboard: 0.05 at 490 nm, and 0.67 at 630 nm; for the green cardboard: 0.55 at 490 nm, and 0.22 at 630 nm). Finally, to get the amount of quanta absorbed by the L- and M-cone type, respectively, these values were weighted by the absorption coefficients of the cone photopigments. Assuming that maximal absorption is equal to 1 for both cone types, we used an absorption coefficient of 0.92 at 490 nm, and 0.00018 at 630 nm for the M-cone type, and the coefficients 0.32 for 490 nm, and 0.33 for 630 nm for the L-cone type.

Table 1 shows the modulation of the M- and L-cone types for each fish when the red and green stripes were illuminated simultaneously by 490 and 630 nm (Table 1 Panel A: 490 nm constant; Table 1 Panel B: 630 nm constant). The mean value of the modulation of the L-cone type at the minimum of the optomotor response for all fish was 1:0.95 (standard deviation: SD = $\pm$0.35), whereas the mean modulation of the M-cone type was with 1:10.96 (SD = $\pm$0.02) much higher.

The data indicate that at the minimum of the optomotor response the L-cone type was hardly modulated at all, while the M-cone type was strongly modulated by the moving red–green cylinder.

4. Discussion

4.1. The optomotor response in zebrafish

Moving striped patterns elicit the optokinetic response, and have been used to screen zebrafish larvae for mutants in which the visual system is affected (Brockerhoff, Dowling, & Hurley, 1998; Brockerhoff et al., 1995; Neuhauss et al., 1999). With a setup very similar to ours, Bilotta (2000) investigated visual acuity in zebrafish in the optomotor response. Optomotor behavior can also be elicited even in larvae by showing moving patterns at the bottom of an elongated tank to investigate motion perception in normal (Orger, Smear, Anstis, & Baier, 2000), and mutant zebrafish (Baier, 2000).

In comparison to the reactions of goldfish, the optomotor response in zebrafish is much more difficult to measure. Probably because of their relatively high swimming speed and spontaneous activity (Fig. 2), their swimming behavior is more variable. As shown in Fig. 3, zebrafish can follow a pattern velocity of up to 20 rpm which corresponds to 120°/s. To obtain useful and reliable data, some changes in setup and procedure were necessary compared to our experiments with goldfish (Schaerer & Neumeyer, 1996). At first, a central post was introduced into the circular test tank to promote movements parallel to the tank wall. Second, reliable results were obtained only after we installed flickerfree fluorescent tubes (70 kHz) above the home tanks, which seems to prevent hectic swimming behavior frequently observed before. Third, startle responses with high swimming speeds irrespective of the direction of pattern movement occurring at the beginning of the pattern movement were excluded from the data by starting to count the swimming behavior only 20 s after movement onset.

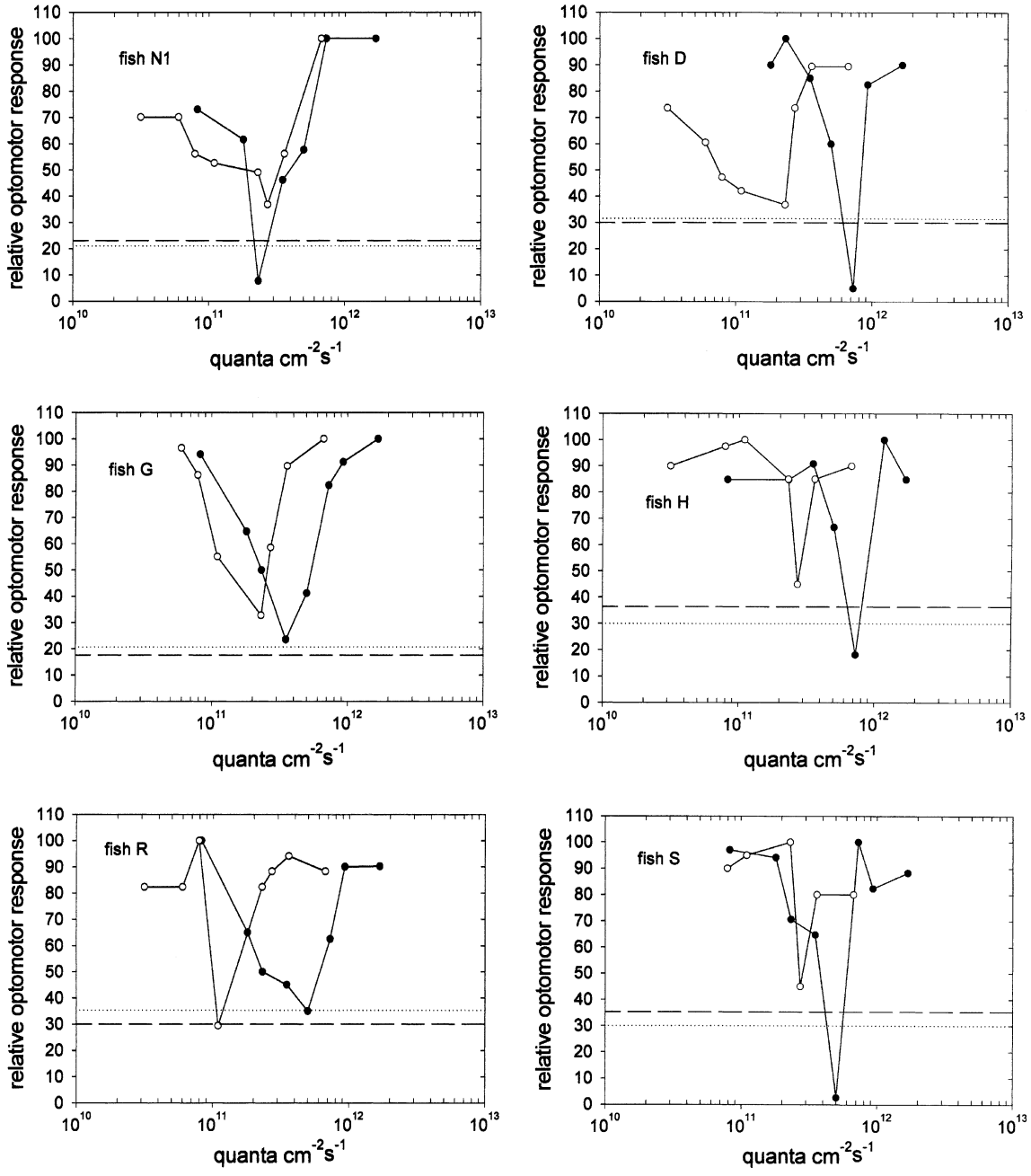

Fig. 7. Relative optomotor response of six fish in the red/green cylinder, simultaneously illuminated with monochromatic light of 490 and 630 nm. Ordinate: the values (given in percent) are related to the maximal optomotor gain of each fish obtained in this measurement. Abscissa: amount of quanta/cm^2 s (measured on the white cardboard) with which the second (variable) wavelength was illuminating the cylinder. Dark symbols: 490 nm set at constant intensity, and the intensity of 630 nm varied according to the abscissa values. Open symbols: 630 nm constant, and 490 nm variable. Dashed line: spontaneous swimming activity in the stationary red/green cylinder illuminated with the constant 490 nm light; dotted line: illumnated with the constant 630 nm light.

4.2. The action spectrum of the optomotor response

The spectral sensitivity values of the five fish tested were maximal in the spectral range between 550 and 600 nm and are, especially in the long wavelength range, similar to the spectral sensitivity of the L-cone type with a maximum at 570 nm as measured in patch clamp recordings by Palacios et al. (1996) in the closely related *Danio aequipinnatus*. As shown in Fig. 5, the long wavelength flank of the L-cone sensitivity coincides well with the behavioral data. This is a good indicator for a contribution of the L-cone type to the action spectrum as there is no other cone type in this wavelength range which could have modified the function. At the short

Table 1
Modulation of the L- and M-cone types by the red/green cylinder, illuminated simultaneously by 490 and 630 nm light

Fish no.	490 nm quanta/cm^2 s	630 nm quanta/cm^2 s	Minimal reaction (% of max. reaction)	Modulation L-cone green:red	Modulation M-cone green:red
Panel A 490 nm constant					
N1	2.71×10^{11}	2.20×10^{11}	8	1:1.20	1:10.98
S	2.71×10^{11}	5.00×10^{11}	20	1:0.73	1:10.95
S	2.71×10^{11}	5.00×10^{11}	2	1:0.73	1:10.95
R	2.71×10^{11}	2.20×10^{11}	47	1:1.20	1:10.98
R	2.71×10^{11}	5.00×10^{11}	35	1:0.73	1:10.95
T	2.71×10^{11}	3.40×10^{11}	23	1:0.91	1:10.97
D	2.71×10^{11}	6.00×10^{11}	5	1:0.67	1:10.94
D	2.71×10^{11}	7.10×10^{11}	5	1:0.62	1:10.93
G	2.71×10^{11}	2.10×10^{11}	10	1:1.24	1:10.98
G	2.71×10^{11}	3.20×10^{11}	23	1:0.94	1:10.97
H	3.60×10^{11}	5.00×10^{11}	29	1:0.86	1:10.96
H	3.60×10^{11}	7.10×10^{11}	19	1:0.71	1:10.95
Panel B 630 nm constant					
N1	2.80×10^{11}	2.32×10^{11}	38	1:1.19	1:10.98
S	2.80×10^{11}	2.32×10^{11}	45	1:1.19	1:10.98
R	1.10×10^{11}	2.32×10^{11}	34	1:0.68	1:10.94
R	1.00×10^{11}	2.32×10^{11}	30	1:0.65	1:10.93
T	2.80×10^{11}	7.27×10^{11}	41	1:0.62	1:10.93
D	7.90×10^{10}	2.32×10^{11}	19	1:0.59	1:10.92
D	2.20×10^{11}	2.32×10^{11}	38	1:1.01	1:10.97
G	2.10×10^{11}	2.32×10^{11}	35	1:0.98	1:10.97
G	2.10×10^{11}	2.32×10^{11}	32	1:0.98	1:10.97
H	7.00×10^{11}	2.32×10^{11}	28	1:2.24	1:10.99
H	2.80×10^{11}	2.32×10^{11}	45	1:1.19	1:10.98

The calculation proceeded from the amount of quanta/cm^2 s of the two monochromatic lights (490 and 630 nm, measured at the white cardboard; left columns) at which the optomotor response was at minimum in Fig. 7. The fourth column gives the minimal values of the optomotor gain, which was in most cases near spontaneous activity. The modulation of the L- and M-cone types, respectively, was calculated taking into account the spectral reflectance of the red and green stripes, and the relative sensitivity of the cone types (see text). In Panel A the intensity of 490 nm was kept constant, in Panel B the intensity of 630 nm. For most fish two measurements were performed under each condition.

wavelength flank of the action spectrum the sensitivity values are in most cases lower than those of the L-cone type. The data scatter in the range of about 1 log unit. A similar distribution of the sensitivity values was found in the corresponding experiment in goldfish (Schaerer & Neumeyer, 1996). In both species we may conclude that the action spectrum of the optomotor response is dominated by the L-cone type. The finding that in three of the five tested zebrafish the sensitivity values in the short wavelength range were lower than the L-cone sensitivity may be due to an inhibitory influence of the M- and/or S-cone type and corresponds to the findings in goldfish (Schaerer & Neumeyer, 1996). As discussed in detail in that paper, we assume that color opponent (R+/G−) ganglion cells may account for the specific shape of the function.

We also measured the action spectrum of the optomotor response in the dark adapted state (data not shown here). The method was the same as in the previous goldfish experiment: after 15 min in the dark at the beginning of the experiment, and no illumination in the pauses between the single trials, an action spectrum with a maximum at about 500 nm was found, which was similar to the rod spectral sensitivity function. In the long wavelength range sensitivity was slightly higher indicating L-cone contribution probably due to incomplete dark adaptation.

4.3. The "color blindness" of the optomotor response

The action spectrum of the optomotor response indicates that mainly one cone type out of the four identified cone types (Nawrocki et al., 1985; Robinson et al., 1993; Palacios et al., 1996) is used for large field motion perception. As a consequence, the animal must be color blind for this task. Thus, the motion of a colored striped pattern which does not modulate the L-cone excitation cannot be perceived. As shown in Fig. 7, there was indeed a reduction of the optomotor response which came close to spontaneous activity, whenever the ratio in the amount of quanta/cm^2 s reflected by the red and green stripes was such that the relative excitation values of the L-cone type were close to 1 (Table 1). The fact that large field motion detection measured in the optomotor response is mediated by one photoreceptor type only, and that this visual ability is "color blind", is now demonstrated in two species of fish, *D. rerio* and *Carassius*

auratus, and in the honey bee *Apis mellifica* (Kaiser & Liske, 1974).

Goldfish and honeybees are species with a highly efficient tetra- and trichromatic color vision, respectively, demonstrated in behavioral training experiments (Neumeyer, 1986, 1992; von Helversen, 1972). The finding that these species behave color blind in the optomotor response indicates a separate processing of color on the one hand, and brightness and motion on the other (Neumeyer, Wietsma, & Spekreijse, 1991). This separate processing seems to be restricted to the contribution of the L-cones. In goldfish, this view is further supported in neuropharmacological investigations, testing wavelength discrimination in training experiments, and motion detection using the optomotor response. Here, a blockade of D1 dopamine receptors in the retina caused red–green color-blindness, but had no effect on the absolute sensitivity for the detection of moving stripes (Mora-Ferrer & Gangluff, 2000, Fig. 3B; Mora-Ferrer & Neumeyer, 1996). In contrast, sulpiride, an inhibitor of D2 dopamine receptors reduced absolute sensitivity in the optomotor response, but had no effect in wavelength discrimination (Mora-Ferrer & Gangluff, 2000, Fig. 3A; Mora-Ferrer & Neumeyer, 1996, Fig. 9).

A separation of color and motion vision also exists in monkeys (Zeki, 1977) and humans (Livingstone & Hubel, 1987, 1988). The existence of these parallel pathways was supported by psychophysical experiments (Cavanagh, Tyler, & Favreau, 1984; Ramachandran & Gregory, 1978). They showed that the perceived velocity of a colored moving stimulus is reduced at isoluminant conditions.

Parallel processing of color and motion realized in species as different as honeybees, cyprinid fishes, and primates indicates a similar selective pressure acting on the visual system. The finding that one photoreceptor type only is contributing to large field motion detection may have the following advantage: in a complex "colorful" world, a high correlation between the two channels of a motion detector is provided only if the channels have the same spectral sensitivity (Srinivasan, 1985).

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